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(b) adding reagents sufficient for a ligase chain reaction and at least two different oligomers to the sample, wherein each of said primers comprise at least 10 contiguous nucleotides identical or exactly complementary to SEQ ID NO:1.

REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment simply adds sequence identifier numbers or corrects the form of such identifiers and provides the sequence listing for sequences previously included in the application. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NO1-14, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 25 of page 2 has been amended as follows:

In a related aspect, the invention provides an isolated polynucleotide that encodes, or is complementary to a sequence that encodes, the CCX CKR polypeptide. In some embodiments the polynucleotide has at least 10, 15, 25, 50 or 100 contiguous bases identical or exactly complementary to SEQ ID NO:1. In various embodiments, the polynucleotide is the full-length sequence of SEQ ID NO:1, encodes a CCX CKR polypeptide of the invention (e.g., having the sequence of SEQ ID NO:2 [SEQ ID:2] or a fragment thereof), or selectively hybridizes under high stringent hybridization conditions to a polynucleotide sequence of SEQ ID NO:1. The polynucleotide of the invention may be operably linked to a promoter. The invention provides recombinant vector (e.g., an expression vector) expressing the CCX CKR polypeptides of the invention. In one aspect, the invention provides a polynucleotide having sequence encoding a polypeptide that has an activity (e.g., a chemokine binding activity) of a CCX CKR polypeptide and which is (a) a polynucleotide having the sequence of SEQ ID NO:1 or SEQ ID NO:3 [SEQ ID NO 3]; or (b) a polynucleotide which hybridizes under stringent conditions to (a); or (c) a polynucleotide sequence which is degenerate as a result of the genetic code to the sequences defined in (a) or (b).

Paragraph beginning at line 3 of page 5 has been amended as follows:

Figure 1 shows the nucleotide sequence for a human CCX CKR (SEQ ID NO:1) [(SEQ. ID NO:1)] and the predicted amino acid sequence of the human CCX CKR polypeptide (SEQ ID NOS:2 and 12-14) [(SEQ. ID NO:2)].

Paragraph beginning at line 7 of page 5 has been amended as follows:

Figure 2 shows the CCX CKR sequence aligned with those of other chemokine receptors, the expression pattern of CCX CKR RNA, and generation of a stable cell line expressing CCX CKR. Figure 2A shows sequence homology of the CCX CKR coding region (SEQ ID NO:2) with other chemokine receptors (SEQ ID NOS:6-9). Figure 2B shows cells and tissues expressing CCX CKR RNA, as analyzed by RT-PCR of cytoplasmic RNA from cultured primary cells and

whole tissues from various organs as indicated. Figure 2C shows a population of transfected HEK-293 cells stably expressing CCX CKR protein containing an N-terminal Flag epitope, comparing intensity of anti-Flag mAb staining relative to wild type HEK293 cells.

Paragraph beginning at line 13 of page 6 has been amended as follows:

Figure 5 shows DNA sequence 5' to the translation start site of the CCX CKR gene (SEQ ID NOS:10 and 11), as determined from a genomic clone.

Paragraph beginning at line 4 of page 17 has been amended as follows:

Typically, the CCX CKR variants are structurally and functionally similar to the CCX CKR allele having the sequence of SEQ ID NO:2 [SEQ. ID. NO:2]. Structural similarity is indicated by, e.g., substantial sequence identity (as defined above), or immunological cross-reactivity. Functional similarity is indicated by, e.g., a ligand-binding specificity similar to or the same as that of the naturally occurring CCX CKR allele CCX CKR allele having the sequence of SEQ ID NO:2 [SEQ. ID. NO:2] (e.g., binding ELC, SLC, and TECK with high affinity). In some embodiments, the CCX CKR polypeptide of the invention is a fusion protein or a fragment (e.g., a ligand binding fragment) of the full-length polypeptide encoded in SEQ ID NO:2 [SEQ. ID. NO:2]. As used in this context, a "ligand binding fragment" of CCX CKR is a fragment of the receptor polypeptide that binds ELC (e.g., human or mouse ELC), SLC (human or mouse), or TECK (human or mouse) with high affinity (e.g., an apparent Ki or relational IC50 of less than about 15 nM) or moderate affinity (e.g., an apparent Ki or relational IC50 of at between about 15 and about 200 nM). Suitable assays for detecting binding are well known in the art. See, e.g., E.C Hulme "Receptor-Ligand Interactions" in A PRACTICAL APPROACH/ THE PRACTICAL APPROACH SERIES (Series Eds D. Rickwood and BD Hames) IRL Press at Oxford University Press (1992), especially Ch. 6, Wang et al., "The se of the filtration technique in *in vitro* radioligand binding assays for membrane-bound and solubilized receptors," and Ch. 7, Hulme et al., "Centrifugation binding assays"; see also, Sissors et al., 1999, "A Homologous Receptor Binding Assay for HTS on FlashPlate plusNEN Life Science Products inc, Boston, MA 02118.

Paragraph beginning at line 17 of page 20 has been amended as follows:

In one aspect, the invention provides a polynucleotide having a sequence or subsequence of a mammalian (e.g., rat or human) CCX CKR gene or RNA. The polynucleotides of the invention (*e.g.*, RNA, DNA, PNA or chimeras), may be single-stranded, double stranded, or a mixed hybrid. In one embodiment, the polynucleotide has a sequence of SEQ ID NO:1 [SEQ. ID NO: 1] (Figure 1) or subsequences thereof (e.g., comprising at least 15, at least 25, at least 50, at least 100, at least 200, or at least 500 bases of the polynucleotides and variants of the invention). The invention also provides polynucleotides with substantial sequence identity to the CCX CKR polynucleotides disclosed herein. Thus, the invention provides naturally occurring alleles of mammalian (e.g., human) CCX CKR genes such as human allelic variants of the CCX CKR polynucleotides of SEQ ID NO:1.

Paragraph beginning at line 27 of page 20 has been amended as follows:

As described *infra*, in some embodiments the polynucleotide of the invention encodes a polypeptide with substantial sequence similarity to SEQ ID NO:2 [SEQ. ID NO:2] (Figure 1) or encodes a fragment of such a polypeptide (e.g., a fusion protein). Also contemplated are polynucleotides that, due to the degeneracy of the genetic code, are not substantially similar to SEQ ID NO:1, but encode the polypeptide of SEQ ID NO:2 [SEQ. ID NO:2] or a fragment thereof. In other embodiments, the invention provides CCX CKR polynucleotides that do not necessarily encode CCX CKR polypeptide but which are useful as *e.g.*, probes, primers, antisense, triplex, or ribozyme reagents, and the like.

Paragraph beginning at line 19 of page 21 has been amended as follows:

In one aspect, the invention provides polynucleotides encoding CCX CKR polypeptides such as an CCX CKR polypeptide having the sequence of SEQ ID NO:2, a fragment thereof, a variant thereof (*e.g.*, a conservative or allelic variant), or a CCX CKR fusion polypeptide. In one embodiment, the polynucleotide of the invention comprises the sequence of SEQ ID NO:1 or a fragment thereof. In another embodiment, the polynucleotide encodes a naturally occurring CCX CKR polypeptide or fragment, but has a sequence that differs from SEQ ID NO:1 [SEQ. ID NO:1] (*e.g.*, as a result of the degeneracy of the genetic code). In some embodiments of the invention, the polynucleotide is other than the expressed sequence tags H67224, AI131555, AA215577, AW190975 or AI769466 or the polynucleotide encoding bovine PPR1 (Matsuoka et al,., 1993, *Biochem Biophys*

Res Comm 194:540-11).

Paragraph beginning at line 26 of page 22 has been amended as follows:

In one embodiment, the invention provides oligonucleotide or polynucleotide probes and/or primers for detecting or amplifying CCX CKR polynucleotides. In various embodiments, the polynucleotides (e.g., probes and primers) comprise at least 10 contiguous bases identical or exactly complementary to SEQ ID NO:1, usually at least 12 bases, typically at least 15 bases, generally at least 18 bases and often at least 25, at least 50, or at least 100 bases When the CCX CKR polynucleotides of the invention are used as probes or primers they are generally less that about 3000 bases in length; typically they contain between about 12 and about 100 contiguous nucleotides identical or exactly complementary to SEQ ID NO:1 [SEQ. ID NO:1], more often between about 12 and about 50 contiguous nucleotides, even more often between about 15 and about 25 contiguous nucleotides.

Paragraph beginning at line 27 of page 24 has been amended as follows:

The antisense nucleic acids (DNA, RNA, modified, analogues, and the like) can be made using any suitable method for producing a nucleic acid, such as the chemical synthesis and recombinant methods disclosed herein. In one embodiment, for example, antisense RNA molecules of the invention may be prepared by *de novo* chemical synthesis or by cloning. For example, an antisense RNA that hybridizes to CCX CKR mRNA can be made by inserting (ligating) an CCX CKR DNA sequence (e.g., SEQ ID NO:1 [SEQ. ID No; 1], or fragment thereof) in reverse orientation operably linked to a promoter in a vector (e.g., plasmid). Provided that the promoter and, preferably termination and polyadenylation signals, are properly positioned, the strand of the inserted sequence corresponding to the noncoding strand will be transcribed and act as an antisense oligonucleotide of the invention. The antisense oligonucleotides of the invention can be used to inhibit CCX CKR activity in cell-free extracts, cells, and animals, including mammals and humans.

Paragraph beginning at line 21 of page 27 has been amended as follows:

Gene therapy refers to the introduction of an otherwise exogenous polynucleotide which produces a medically useful phenotypic effect upon the (typically) mammalian cell(s) into which it is transferred. In one aspect, the present invention provides gene therapy methods and

compositions for treatment of CCX CKR-associated conditions. In illustrative embodiments, gene therapy involves introducing into a cell a vector that expresses an CCX CKR gene product (such as an CCX CKR protein substantially similar to the CCX CKR polypeptide having a sequence of SEQ ID NO:2 [SEQ. ID NO:2], e.g., to increase CCX CKR activity, or an inhibitory CCX CKR polypeptide to reduce activity), expresses a nucleic acid having an CCX CKR gene or mRNA sequence (such as an antisense RNA, e.g., to reduce CCX CKR activity), expresses a polypeptide or polynucleotide that otherwise affects expression of CCX CKR gene products (e.g., a ribozyme directed to CCX CKR mRNA to reduce CCX CKR activity), or replaces or disrupts an endogenous CCX CKR sequence (e.g., gene replacement and gene knockout, respectively). Numerous other embodiments will be evident to one of skill upon review of the disclosure herein.

Paragraph beginning at line 3 of page 54 has been amended as follows:

--BLAST analysis of known chemokine receptors identified a bovine receptor, PPR1, designated as a gustatory receptor (Matsuoka et al,., 1993, *Biochem Biophys Res Comm* 194:540-11). A search of a human EST database using the PPR1 sequence identified two non-contiguous EST's: H67224 and AI131555. Primers were designed against the 5' end of H67224 (5' AAT TTG GCT GTA GCA GAT TTA CTC C 3' [SEQ ID NO:4] [[SEQ. ID. NO:4]]) and in the reverse orientation for the 3' end of AI131555 (5' GCT AAA AGT ACT GGT TGG C 3'[SEQ ID NO:5] [[SEQ. ID. NO:5]]), and used in PCR (5% DMSO, annealing 58°C) of genomic DNA isolated from human buffy coats. The reaction resulted in a 855 bp product containing the ESTs and connecting sequences. The 855 bp fragment product was used to design additional primers for use in an anchored PCR screen of a Rapid Screen™ arrayed spleen cDNA library (Origene, Rockville, MD), yielding a 5' extended clone; this clone was finally used to screen a human genomic library by filter hybridization. Full length coding sequence was deduced by sequence analysis of genomic clones using reverse primer from the 5' sequence of Origene clone PCR with proofreading Pfu (Stratagene) enzyme. The refined sequence was confirmed on several clones and is shown in Figure 1. [A preliminary sequence determination differed from Figure 1 at the following positions: 47, 64, 78, 120, 131, 545, 571, 574 (using the numbering of Figure 1) which were G, G, G, C, C, T, A, and T, respectively [SEQ ID NO:3] [[SEQ. ID. NO:3]], which variant is also contemplated by the invention). The coding sequence was cloned into pIRESpuro expression vector (Clontech, Palo Alto, CA) with a FLAG epitope tag and prolactin signal sequence.

In the Claims:

Claim 24 has been amended as follows:

24. A method of amplifying a CCX CKR polynucleotide in a sample comprising

(a) adding reagents sufficient for a polymerase chain reaction and at least two different primers to the sample, wherein each of said primers comprise at least 10 contiguous nucleotides identical or exactly complementary to SEQ ID NO:1 [SEQ. ID. NO:1]; or,

(b) adding reagents sufficient for a ligase chain reaction and at least two different oligomers to the sample, wherein each of said primers comprise at least 10 contiguous nucleotides identical or exactly complementary to SEQ ID NO:1 [SEQ. ID. NO:1].